

The University of Chicago

# THE PRODUCTION OF TUMORS BY TOBACCO TARs

A DISSERTATION SUBMITTED TO THE  
FACULTY OF THE DIVISION OF THE  
BIOLOGICAL SCIENCES IN CANDIDACY FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY

1940

By

CURTIS M. FLORY

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# The Production of Tumors by Tobacco Tars

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The main purpose of this paper is to report the results of an investigation of the carcinogenic effects of destructive distillates of tobacco and of pipe tar. It is concerned chiefly with the experimental production of benign and malignant tumors in rabbits, rats, and mice painted or injected with tobacco tars. To give the background, a review will be presented of some of the clinical studies which have indicated that the smoking of tobacco is a factor in the etiology of carcinoma of the lip, the tongue and oral cavity, the pharynx and larynx, and the lung in man. Experimental studies of the carcinogenic action of various tars will also be reviewed.

Carcinoma of the lip, formerly referred to as "pipe-smoker's cancer," has been said by Ewing (12) to be traceable in many cases to the irritation of a pipe or cigar, or both. Broders (4) found that three-fourths of the smokers with this disease smoked pipes. Other factors, such as sunlight, are also etiologically important.

Almost all of Bloodgood's (2) patients with carcinoma of the tongue used tobacco. Abbe (1) showed that all save one of 90 men with oral cancer were heavy smokers. In 1,413 patients with cancer of the palate, tongue, mouth, and lung, Hoffman (16) found a higher percentage of heavy smokers than in a similar number of controls. He (17) stated that pipe smoking has a direct relationship to cancer of the lip and tongue, and concluded that smoking increases the liability to cancer of the mouth, throat, esophagus, larynx, and lung. Ewing (12) has written that tobacco has "a predominant influence in the development of cancer of the buccal mucosa." He included tobacco as a contributing factor in cancer of the larynx, esophagus, and lung.

The increased use of cigarettes and the habit of inhaling smoke deep into the lungs, has been blamed by many for the increased incidence of carcinoma of the lung. Müller (28) found a high percentage of heavy smokers among males with primary carcinoma of the lung. Lickint (23, 24) stated that smoking was the chief cause of carcinoma of the lung. Simonds (51) placed tobacco smoke as among the possible causes of this tumor, but did not consider it an important etiological factor.

It is not unlikely that a carcinogenic hydrocarbon could be formed during the smoking of tobacco. During smoking tobacco is subjected first to a mild destructive distillation process, then to combustion at temperatures of from 370-700° C. (11). Carcinogenic substances have been formed from other organic materials by similar processes at similar temperatures. Extracts of coal soots are carcinogenic (8). Kennaway (18) has found slight carcinogenic activity in tars produced by the destructive distillation of coal at temperatures as low as 450° C. Tars produced from coal heated to the temperature found in a briar pipe (11), 560° C., were almost as carcinogenic as those produced at 1,250° C. Tars from acetylene heated to 700° C., and isoprene heated to 720° C., the upper limit of pipe tempera-

tures, were carcinogenic (18). At higher temperatures the destructive distillates of petroleum, human skin, and yeast (18), cholesterol (19), turpentine (56), rice polishings and crude sugar (20), all were shown to be carcinogenic.

Tobacco, like many of the above substances, is a complex mixture of chemicals, and by subjecting it to combustion and destructive distillation processes many workers have attempted to obtain carcinogenic substances.

In Table I are listed the attempts to produce carcinomas with tobacco products. The later work of Roffo and that of Sugiura are discussed separately.

In 1936 Roffo (34, 35) reported the results of painting the destructive distillates of Kentucky tobacco daily on the ears of rabbits. The watery 0-120° C. fraction produced no tumors in 10 months. The 120-350° C. fraction, a tar, produced papillomas in most animals in 7 months, and squamous cell carcinomas in all in 9 months. The residual tar also produced carcinomas, but not as frequently. Spectroscopy suggested the presence of 1,2-benzanthracene. However, further spectroscopic work (36) on this and other tobacco tars showed only the presence of condensed benzenic nuclei.

Roffo (37) obtained similar results with Egyptian and Turkish tobaccos. He (38, 39, 43) subsequently used 9 varieties of tobacco, painting the rabbits every other day with a 120-350° C. destructive distillate. The first papilloma was seen in 26 days, and many tumors developed between 40 and 50 days. Each of the tars produced tumors in most of the animals. Many of these tumors seem to have been papillomas, but he stated that 10 rabbits painted with tar from Egyptian tobacco developed squamous cell carcinomas in from 8 to 14 months.

In 1939 Roffo (40, 41) reported spectroscopic studies of the destructive distillates of tobacco distilled at 380° C. A similarity was found in the absorption bands of tobacco tar and of 1,2-benzpyrene between the ranges of 2100 Å. and 3100 Å. His conclusion that the distillate contained 1,2-benzpyrene would be more understandable if he had demonstrated strong bands with their lower edges at 4040 Å. and 4270 Å. It is in this region of the spectrum that Hieger (14) and others (26) have located the bands of 3,4-benzpyrene. (Formerly numbered 1,2-benzpyrene (19).)

Recently Roffo (42) prepared tars by the combustion of tobacco in a large pipe. He condensed the smoke in water and in cotton filters, extracted the tar, and painted rabbits' ears every other day. The first papilloma was seen at 49 days, and 8 rabbits developed tumors by 290 days. At 12 months each rabbit had from 6 to 13 tumors, some of which were described as squamous cell carcinomas.

In 1940 Sugiura (52) heated leaf tobaccos in an iron still at from 100-500° C., then at from 500-900° C., collecting the products of each distillation. Groups of mice were painted twice weekly with the distillates. Only 1 mouse developed a squamous cell carcinoma. This appeared on a mouse painted for 315 days with the 500-900° C. tar. Using an oily mixture of both distillates he painted the ears of rats for periods up to 65 weeks without effect, and also painted the ears of rabbits 3 times a week for from 52 to 95 weeks without tumor production.

TABLE I: ATTEMPTS TO PRODUCE CARCINOMAS WITH TOBACCO PRODUCTS

| Year | Author                              | Substance used                                                                                 | Animals     | Method                    | Time *    | Results                                             |
|------|-------------------------------------|------------------------------------------------------------------------------------------------|-------------|---------------------------|-----------|-----------------------------------------------------|
| 1900 | Brosch (5)                          | Tobacco juice                                                                                  | Guinea pigs | Painting                  | ?         | Proliferation                                       |
| 1911 | Wacker and Schmincke (54, 55).      | Pipe tars                                                                                      | Rabbits     | Injection                 | 21 D      | Proliferation                                       |
| 1923 | Hoffman, Schreus and Zurhelle (15). | Tobacco tar denicotinized.                                                                     | Mice        | Painting                  | 14 D      | Hairlessness                                        |
| 1928 | Helwig (13)                         | Pipe tar                                                                                       | Rabbits     | Injection                 | 4 M       | Proliferation                                       |
|      |                                     | Nicotine free ether extract of pipe tar.                                                       | Mice        | Painting                  | 1 Y       | Benign ulcers                                       |
|      |                                     | 400-500° C. destructive distillate of tobacco.                                                 | Mice        | Painting                  | 8 M       | Benign ulcers                                       |
| 1930 | Roffo (29)                          | Tobacco smoke                                                                                  | Rabbit      | Blowingsmoke              | .....     | Oral leukoplakia.                                   |
|      | Roffo (30, 31)                      | Condensed tobacco smoke in water.                                                              | Rabbit      | Painting                  | 8 M       | Papillary carcinoma on ear                          |
| 1931 | Chickamatsu (9)                     | Tobacco tar                                                                                    | Rabbit      | Painting                  | 225 D     | Cancroid of ear                                     |
| 1931 | Cooper, Lamb, and Sanders (11).     | Tar from pipe smoke, cold, hot, and in various solvents.                                       | Mice        | Painting                  | 16 M      | 1 carcinoma                                         |
| 1932 | Roffo (33, 32)                      | Jet of tobacco smoke                                                                           | Rabbits     | Smoke on ear              | 3 Y       | 1 carcinoma with cervical metastases                |
|      | Roffo (34)                          | Pills of tobacco tar                                                                           | Rats        | Implants                  | 8 M       | Neoplasm-like tumors                                |
|      | Moratti (27)                        | Cigars                                                                                         | Dog         | Inserted in gall bladder. | .....     | Chronic inflammation                                |
|      |                                     | Nicotine solution                                                                              | Dog         | Injected in breast.       | .....     | Chronic inflammation                                |
|      | Bogen and Loomis (3)                | Tar from the destructive distillation of tobacco, denicotinized.                               | Mice        | Painting                  | 1 Y       | No effect                                           |
|      |                                     |                                                                                                | Rabbits     | Painting                  | 6 M       | No effect                                           |
|      | McNally (25)                        | Tobacco tar                                                                                    | Rats        | .....                     | .....     | Too toxic                                           |
|      |                                     | Water solution of cigarette smoke.                                                             | Rats        | Spraying                  | 100 D     | No effect                                           |
|      | Lu-Fu-Hua (21, 22)                  | Tar from burning tobacco on 1 ear, plus some coal tar on other ear.                            | Rabbits     | Painting                  | 148-182 D | 3 cancers on tobacco ear                            |
|      |                                     | Tobacco tar plus injected cholesterol.                                                         | Rabbits     | Painting                  | 205 D     | 1 cancer                                            |
| 1935 | Schürch and Winterstein (47).       | Denicotinized tar from cigar smoke.                                                            | Mice        | Painting                  | 258-635 D | No tumors                                           |
|      |                                     | Distillates of above cigar tar.                                                                | Mice        | Painting                  | 60-480 D  | No tumors                                           |
|      |                                     | Cigar tar                                                                                      | Rabbits     | Painting ears.            | 223 D     | No effect                                           |
|      |                                     |                                                                                                |             | Painting mouth            | 548 D     | No effect                                           |
|      |                                     | Coal tar to one ear until warts appear, ear amputated. Other ear painted with cigar smoke tar. | Rabbits     | Painting                  | 918 D     | Carcinoma in 1 rabbit at 344 D, warts in 6 at 918 D |
| 1937 | Schürch and Winterstein (48).       | As above                                                                                       | Rabbits     | Painting                  | 48 M      | Carcinoma in 1 rabbit, warts in 2                   |
| 1936 | Campbell (7, 8)                     | Cigarette smoke tar                                                                            | Mice        | Painting                  | 16 M      | 1 carcinoma                                         |
| 1937 | Taki (See Kinoshita (20).           | Tar from tobacco pipes                                                                         | Mice        | Painting                  | 130 D     | 2 carcinomas; 3 papillomas                          |

\* D = days; M = months; Y = years.



## EXPERIMENTAL

## MATERIALS AND METHODS

*Tobacco used.*—The tobacco used was a cheap grade of cured Kentucky tobacco. The entire leaf and leaf stem were used.

*Temperature determinations.*—Tobacco smoked slowly in briar pipes burned at from 425-560° C. A few puffs raised the temperature above 600° C., and

The still was filled with 2.5 kg. of dry tobacco; the asbestos gasket, soaked in water-glass (liquid sodium silicate), put in place; and the lid fastened tightly so as to prevent the escape of fumes. The still was then slowly heated to 700° C. Six to 8 hours were required for a distillation. A straight-tubed water-cooled condenser leading into a filter flask was used to collect the water and tar, and the noncondensable products were led into a hood.

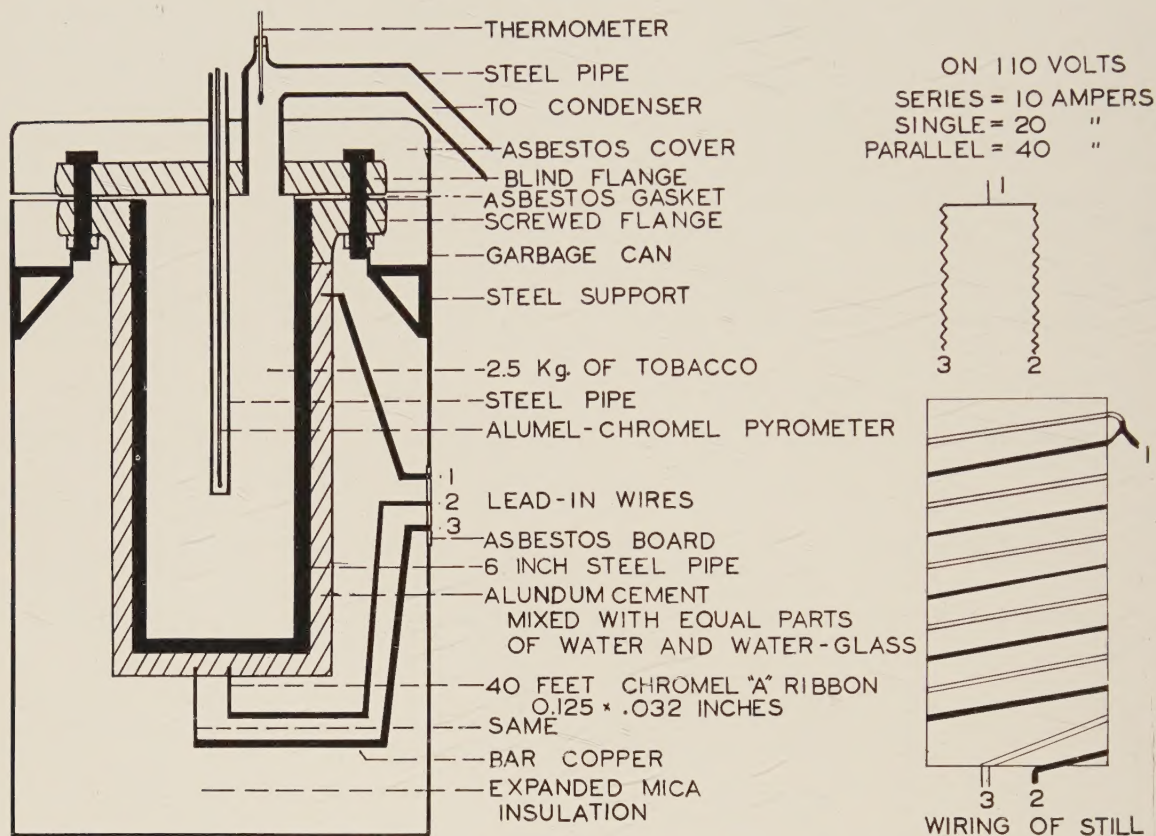


FIG. 1.—The electrically heated still used in preparing the destructive distillates of tobacco.

on several occasions almost to 700° C. Temperatures of 645-704° C. were measured in clay pipes during puffs, and on one occasion reached 750° C. During puffs cigarettes burned at from 604-670° C. Matches burned at from 850° to almost 1000° C. These findings agree with those of Cooper, Lamb, and Sanders (11).

All temperatures were measured by alumel-chromel thermocouples, made of number 28 wire, and calibrated against a standardized alumel-chromel thermocouple. The determinations were accurate to within 12° C.

*Preparation of the destructive distillates of tobacco.*—The destructive distillates were prepared in an electrically heated steel still, shown in Fig. 1.

The 0-120° C. fraction was taken when a thermometer in the still outlet reached 120° C. This fraction consisted largely of water. Any oily material which came over was added to the next fraction. The second, the 120-350° C. fraction, included all condensable material obtained before an alumel-chromel pyrometer, placed in the center of the tobacco in the still, registered 350° C. This fraction consisted of approximately equal layers of an oily black liquid and a heavier, brownish, watery liquid. This mixture was reheated and all material boiling below 120° C. removed. The remaining black, homogenous, tarry substance is called the 120-350° C. destructive distillate tar. The third fraction was taken from 350-700° C. as measured by the pyrometer. This distillate consisted



of thick tarry material and a little water which was removed in a separatory funnel. All residue in the condenser was added to this tar, which is called the 350-700° C. destructive distillate tar.

For each kg. of tobacco distilled, the 0-120° C. fraction yielded 3 gm. of tar (which were added to the 120-350° C. fraction) and 131 gm. of watery material; the 120-350° C. fraction yielded 90.2 gm. of tarry material, and 95 gm. of watery material boiling under 120° C.; and the 350-700° fraction contained 27.5 gm. of tar, and 8.4 gm. of watery material. The amount of the 120-350° tar was over 3 times as great as that of the 350-700° C. tar.

*Preparation of pipe tar.*—Tobacco was coarsely ground and burned in small clay pipes. The pipe stems were passed through small corks, and these inserted in the ends of 6 small steel pipes threaded into the side of a larger steel pipe. From one end of this large pipe the smoke passed down through water in a partly filled liter flask, through a liter cylinder filled with glass wool, and then through a second flask containing water. Continuous suction was furnished by a water pump. The pipes were lighted with wooden matches. Six pipes were usually burned at one time. In this apparatus the tobacco burned at the temperatures found in the briar and clay pipes during puffs, from 520-730° C. as measured by a thermocouple.

After from 1 to 2 kg. of tobacco were burned, the apparatus, the pipes excepted, was freed of tar by washing first with water, then with ether. The combined water in the apparatus and the water and ether from the washings were evaporated at reduced pressure at 70° C. A thin tar was obtained, called pipe tar, parts of which solidified at room temperature. An average of 113 gm. of tar was obtained from 1 kg. of tobacco. This tar is probably similar, at least qualitatively, to what could be obtained from ordinary pipe smoke.

*Animals and methods.*—Rabbits, mice, and rats were used as test animals. The rabbits were mostly domestic albinos, and were obtained from three different dealers. No difference in the reactions of these rabbits to tobacco tars could be attributed to their strain or color. The mice were all white. They were not of an inbred strain, but were bred from a small colony in this laboratory. No spontaneous squamous cell carcinomas have been seen in about 2,000 of these mice, some observed in other experiments for as long as 2 years. The rats were albinos, from a stock bred in this laboratory.

All mice and rabbits were painted 5 times a week, the mice on the back, the rabbits on the inner surface of the right ear. In all rabbits where tumors were studied histologically the subauricular lymph nodes and lungs were similarly examined, and frequently other viscera as well. Multiple sections were made of all subauricular nodes.

Certain mice were painted with a denicotinized tar.

The crude tar was shaken twice with equal parts of 2 per cent HCl solution, draining off the watery liquid each time, then washed of the excess acid with an equal part of distilled water. This treated tar is called denicotinized tar.

*Control studies with coal tar.*—No extensive work was done on rabbits to determine the time of production of carcinomas by coal tar. However a few animals were painted with a coal tar of known carcinogenic potency in order to obtain tar tumors for histological comparisons. The inner surfaces of the right ears of 4 rabbits were painted 5 times a week with coal tar dissolved in equal parts of benzene. Papillomas first appeared in from 42 to 159 days. One rabbit was killed at 188 days, the rest at 525 days. Nineteen tumors were examined histologically, and all were benign papillomas.

Fifteen mice were painted on the back of the neck 5 times a week with the same benzene solution of coal tar. The first papilloma was seen at 107 days, and the first squamous cell carcinoma of the skin at 126 days. In all, 13 mice developed these carcinomas, the last at 256 days. Three of these squamous cell carcinomas metastasized to the lungs, another to a lymph node. Obviously the mice used in these experiments were relatively susceptible to tar painting.

*Classification of rabbit tumors.*—In this work the rabbit tumors have been classified as papillomas and carcinomatoid tumors. The papillomas are the same kind of wart-like growths Yamagiwa and Ichikawa (57) called "folliculo-epitheliomata," and Rous and Kidd (44) common papillomas.

The carcinomatoid tumors require more explanation. Rous and Kidd (44) divided coal tar-induced rabbit tumors into four groups: common papillomas, frill horns, carcinoids, and carcinomas. No frill horns were noted in our animals. They described carcinoids as tumors which had the morphology of squamous cell carcinomas, yet vanished or assumed a benign form when tarring was left off. At first these tumors were markedly invasive, with strands of individual cells growing down into the connective tissue. Grossly they were "raised, raw, ragged disks with infiltrating edges . . . or else raised discoid ulcers." Occasionally they penetrated the lacunae in the cartilages to the other side of the ear. Frequently strands of cells grew directly into the local lymphatics, yet Rous and Kidd never observed a metastasis to a distant site. With continued tarring these tumors eventually took on gross forms like those of old papillomas, or else retrogressed. When tarring was stopped the carcinoids apparently retrogressed or became papillomas. All attempts to transplant carcinoids failed.

Recently (45) Rous and Kidd have suggested that these tumors be called carcinomatoids to avoid confusion with the argentaffine tumors of the human in-



testinal tract. In this paper the term carcinomatoid has accordingly been used.

Extreme anaplasia of a locally invasive tumor may raise the question of malignancy, but not establish it beyond question. The only true criterion for a carcinoma of the ear of a rabbit is a metastasis to a lymph node or other distant site, or a tumor that is transplantable. Ulceration, local destruction of the ear, and

TABLE II: GRAMS OF TAR PAINTED ON EACH RABBIT

| Tars                                   | Per painting  |            | Total for year |
|----------------------------------------|---------------|------------|----------------|
|                                        | First 70 days | First year |                |
| 350-700° C. destructive distillate tar | 0.38          | 0.56       | 146.0          |
| 120-350° C. destructive distillate tar | 0.34          | 0.38       | 98.5           |
| Pipe tar                               | 0.31          | 0.32       | 84.0           |

The earliest time at which a papilloma was seen was on the 37th day in a rabbit painted with pipe tar. The mean time of appearance of the papillomas was, however, the best guide to the papilloma-producing action of the tars, and this mean time was 57 days for the 350-700° C. tar, 75 days for the 120-350° C. tar, and 108 days for the pipe tar. After the appearance of the papillomas drawings were made of the ears at monthly intervals to make records of the time of appearance, number, and character of the tumors. In Tables III, IV, and V are shown the number of rabbits painted with each tar, their color, the days on which the rabbits first developed tumors, the increase in number and kinds of tumors examined histologically, and the present status of the rabbits. Table VIII is a summary of all the tumors produced in rabbits.

As can be seen from these tables, the 350-700° C. tar

TABLE III: PRODUCTION OF TUMORS BY THE 350-700° C. DESTRUCTIVE DISTILLATE TOBACCO TAR

| Rabbit     | Color         | Tumor first seen, days | Number of tumors (mostly papillomas) seen<br>R = Regression of all tumors |     |     |     |     |     |     |     |     |     |     |     | Tumors examined histologically |                     | Status of animals at indicated days |
|------------|---------------|------------------------|---------------------------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------------------------|---------------------|-------------------------------------|
|            |               |                        | Days                                                                      |     |     |     |     |     |     |     |     |     |     |     | Papil-<br>lomas                | Carci-<br>nomatoids |                                     |
|            |               |                        | 50                                                                        | 100 | 150 | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 |                                |                     |                                     |
| 21         | White.....    | 49                     | 5                                                                         | 7   | 8   | 8   | 6   | 6   | 10  | 12  | 8   | 8   | 8   | 6   | 6                              | 1                   | Alive 660                           |
| 22         | White.....    | 50                     | 3                                                                         | 6   | 7   | 5   | 5   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 7                              | ..                  | Died 265                            |
| 23         | White.....    | 72                     | ..                                                                        | 3   | 5   | 1   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1                              | ..                  | Died 238                            |
| 24         | White.....    | 64                     | ..                                                                        | 6   | 13  | 12  | 12  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 15                             | ..                  | Died 272                            |
| 25         | White.....    | 49                     | 3                                                                         | 6   | 11  | 11  | 11  | 9   | 13  | 14  | 14  | 8   | ..  | ..  | 3                              | 1                   | Died 590                            |
| 26         | White.....    | 72                     | ..                                                                        | 4   | 4   | 7   | 8   | 6   | 7   | 12  | 11  | 11  | ..  | ..  | 7                              | ..                  | Died 540                            |
| 27         | White.....    | 66                     | ..                                                                        | 2   | 3   | 4   | 4   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 4                              | ..                  | Died 268                            |
| 28         | White.....    | 57                     | ..                                                                        | 8   | 6   | 7   | 8   | 9   | 10  | 7   | 9   | 3   | 3   | ..  | 2                              | ..                  | Died 585                            |
| 29         | White.....    | 57                     | ..                                                                        | 5   | 3   | 7   | 6   | 6   | 7   | 11  | 10  | 6   | ..  | ..  | 1                              | 2                   | Died 534                            |
| 30         | White.....    | 49                     | ..                                                                        | 2   | 8   | 13  | 8   | 9   | 9   | 16  | 11  | 10  | ..  | ..  | 19                             | 1                   | Died 521                            |
| 31         | Black-white.. | 74                     | ..                                                                        | 2   | 2   | 1   | 2   | 1   | R   | R   | R   | R   | R   | R   | ..                             | ..                  | Alive 660                           |
| 32         | Black-white.. | 79                     | ..                                                                        | 2   | 4   | 4   | 5   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 3                              | ..                  | Died 289                            |
| Total..... |               |                        |                                                                           |     |     |     |     |     |     |     |     |     |     |     | 68                             | 5                   |                                     |

local lymphatic vessels may all occur in carcinomatoid tumors.

*Effects of tobacco tars on rabbits.*—The 350-700° C. destructive distillate tar, the 120-350° C. destructive distillate tar, and the pipe tar were each painted 5 times a week on the inner surfaces of the right ears of rabbits. The quantities of the tars used are shown in Table II.

The increase in quantity of the 350-700° C. tar painted was required to cover the papillomas which appeared.

*Production of papillomas.*—The 350-700° C. tar caused rapid thickening of the skin at the painting site, and after the first week thin sheets of loose skin could be peeled from the ears. From then on the ears were peeled of loose skin about twice a week to permit direct application of the tar to the skin. After 30 days hyperkeratosis of the hair follicles was noted, and rapidly increased. The last of the hair disappeared at about this time.

The ears of the rabbits painted with the other 2 tars showed similar, but less marked changes.

produced the most tumors. The average rabbit painted with this tar developed a maximum of 9 papillomas. The 120-350° C. tar produced an average of only 4.3 tumors, and the pipe tar 4.7 tumors per rabbit.

Despite this greater average production of tumors by the 350-700° C. tar, it is evident from the tables that there was great variation in papilloma production among the rabbits of each series. Some rabbits in each series developed a large number of papillomas, some few or none. It is evident that the variation of susceptibility of the individual animals was greater than the difference in potency of the tars.

The size and shape of the papillomas also varied. Some of the papillomas appeared as low warts 6 or 8 mm. across, growing rapidly into large stalked tumors 2 or 3 cm. in diameter and height. Others never grew over a few mm. high and retained a broad base.

The early papillomas were composed largely of thick hyperkeratotic epithelium projecting up from the ear in filiform processes. As the tumors became larger the



Number of tumors (mostly papillomas) seen  
R = Regression of all tumors

| Rabbit number | Color         | Tumor first seen, days | R = Regression of all tumors |     |     |     |     |     |     |     |     |     |     |     | Tumors examined histologically |                 | Status of animals at indicated days |     |
|---------------|---------------|------------------------|------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------------------------|-----------------|-------------------------------------|-----|
|               |               |                        | Days                         |     |     |     |     |     |     |     |     |     |     |     | Papil-lomas                    | Carci-nomatoids |                                     |     |
|               |               |                        | 50                           | 100 | 150 | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 |                                |                 |                                     |     |
| 1             | White.....    | 93                     | ..                           | 1   | 1   | 2   | 2   | 4   | 4   | 5   | 7   | 9   | 6   | ..  | 4                              | ..              | Died                                | 555 |
| 2             | White.....    | 63                     | ..                           | 6   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 6                              | 2               | Died                                | 127 |
| 3             | White.....    | 68                     | ..                           | 3   | 5   | 4   | 4   | 7   | 6   | 5   | ..  | ..  | ..  | ..  | 1                              | ..              | Died                                | 439 |
| 4             | White.....    | 190                    | ..                           | ..  | ..  | 1   | 2   | 2   | 1   | 2   | ..  | ..  | ..  | ..  | 2                              | ..              | Died                                | 422 |
| 5             | White.....    | 50                     | 1                            | 1   | 2   | 2   | 4   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 3                              | ..              | Died                                | 299 |
| 6             | White.....    | 73                     | ..                           | 3   | 3   | 2   | 2   | 2   | 2   | ..  | ..  | ..  | ..  | ..  | 1                              | 3               | Died                                | 376 |
| 7             | White.....    | ..                     | ..                           | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..                             | ..              | Died                                | 23  |
| 8             | White.....    | 68                     | ..                           | 1   | 1   | 3   | 3   | 1   | ..  | ..  | ..  | ..  | ..  | ..  | 1                              | ..              | Died                                | 321 |
| 9             | White.....    | 89                     | ..                           | 2   | R   | 1   | 1   | R   | 1   | 1   | 3   | 2   | ..  | ..  | 4                              | ..              | Died                                | 510 |
| 10            | White.....    | 75                     | ..                           | 5   | 5   | 6   | 6   | 7   | 6   | 11  | 11  | 8   | 9   | 8   | 5                              | ..              | Died                                | 625 |
| 11            | White.....    | 50                     | 1                            | 2   | 1   | R   | 1   | 1   | 3   | 4   | 10  | 11  | ..  | ..  | 3                              | ..              | Died                                | 528 |
| 12            | White.....    | 137                    | ..                           | ..  | 3   | 2   | 3   | 2   | 3   | 2   | ..  | ..  | ..  | ..  | 3                              | ..              | Died                                | 434 |
| 13            | Black-white.. | 142                    | ..                           | ..  | 1   | R   | R   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..                             | ..              | Died                                | 286 |
| 14            | White.....    | 344                    | ..                           | ..  | ..  | ..  | ..  | ..  | 1   | 2   | 2   | R   | 1   | R   | ..                             | ..              | Alive                               | 660 |
| 15            | Black-white.. | 117                    | ..                           | ..  | 3   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1                              | ..              | Died                                | 150 |
| 16            | White.....    | 73                     | ..                           | 2   | 1   | 4   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 6                              | ..              | Died                                | 264 |
| 17            | Grey.....     | 104                    | ..                           | ..  | 1   | 1   | 1   | R   | 2   | 1   | 1   | ..  | ..  | ..  | 1                              | ..              | Died                                | 497 |
| Total.        |               | ..                     | ..                           | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 40                             | 5               |                                     |     |

Number of tumors (mostly papillomas) seen  
R = Regression of all tumors

| Rabbit<br>number | Color      | Tumor<br>first<br>seen,<br>days | R = Regression of all tumors |     |     |     |     |     |     |     |     |     |     |     |    | Tumors examined<br>histologically |                     | Status of animals<br>at indicated days |  |
|------------------|------------|---------------------------------|------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----------------------------------|---------------------|----------------------------------------|--|
|                  |            |                                 | Days                         |     |     |     |     |     |     |     |     |     |     |     |    | Papil-<br>omas                    | Carci-<br>nomatoids |                                        |  |
|                  |            |                                 | 50                           | 100 | 150 | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 |    |                                   |                     |                                        |  |
| Series I         |            |                                 |                              |     |     |     |     |     |     |     |     |     |     |     |    |                                   |                     |                                        |  |
| 41               | Grey.....  | 62                              | ..                           | 2   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1  | 1                                 | Died                | 135                                    |  |
| 42               | Grey.....  | ..                              | ..                           | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | .. | ..                                | Died                | 95                                     |  |
| 43               | Grey.....  | 49                              | 1                            | 1   | 1   | 2   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 2  | ..                                | Died                | 211                                    |  |
| 44               | White..... | 37                              | 1                            | R   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1  | ..                                | Died                | 132                                    |  |
| 45               | White..... | 111                             | ..                           | ..  | 1   | 1   | 2   | 1   | 1   | R   | ..  | ..  | ..  | ..  | 1  | ..                                | Died                | 411                                    |  |
| 46               | White..... | 305                             | ..                           | ..  | ..  | ..  | ..  | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1  | ..                                | Alive               | 640                                    |  |
| 47               | White..... | 52                              | ..                           | 1   | R   | R   | R   | 1   | 1   | 5   | 4   | 5   | 4   | 2   | 2  | ..                                | Alive               | 640                                    |  |
| 48               | White..... | 113                             | ..                           | ..  | 1   | 2   | 2   | 1   | 1   | 2   | 4   | 4   | 4   | 5   | 2  | ..                                | Alive               | 640                                    |  |
| 49               | White..... | 374                             | ..                           | ..  | ..  | ..  | ..  | ..  | 1   | 2   | 3   | 4   | 3   | ..  | 1  | ..                                | Alive               | 640                                    |  |
| 50               | Tan.....   | 111                             | ..                           | ..  | 3   | 9   | 10  | 7   | 8   | 12  | 16  | 11  | 9   | 12  | 4  | ..                                | Alive               | 640                                    |  |
| 51               | White..... | 119                             | ..                           | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1  | ..                                | Died                | 131                                    |  |
| 52               | White..... | 132                             | ..                           | ..  | 1   | 1   | 1   | 2   | 4   | 2   | 1   | 2   | 6   | 5   | 4  | ..                                | Alive               | 640                                    |  |
| 53               | White..... | 94                              | ..                           | 1   | 1   | 1   | 1   | 3   | 5   | 7   | 6   | 5   | 4   | 5   | 3  | ..                                | Alive               | 640                                    |  |
| Series II        |            |                                 |                              |     |     |     |     |     |     |     |     |     |     |     |    |                                   |                     |                                        |  |
| 54               | White..... | 188                             | ..                           | ..  | ..  | 1   | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | 1  | 1                                 | Died                | 232                                    |  |
| 55               | White..... | 247                             | ..                           | ..  | ..  | ..  | 1   | 2   | 2   | 4   | 3   | ..  | ..  | ..  | 1  | ..                                | Alive               | 500                                    |  |
| 56               | White..... | 107                             | ..                           | ..  | 1   | R   | 2   | 1   | 1   | 1   | ..  | ..  | ..  | ..  | 1  | ..                                | Killed              | 418                                    |  |
| 91               | White..... | 89                              | ..                           | 1   | 2   | 3   | 4   | 4   | 5   | 4   | ..  | ..  | ..  | ..  | 1  | ..                                | Alive               | 440                                    |  |
| 92               | White..... | 99                              | ..                           | 1   | 2   | 2   | 5   | 7   | 8   | 10  | ..  | ..  | ..  | ..  | 1  | ..                                | Alive               | 440                                    |  |
| 93               | White..... | 99                              | ..                           | 1   | 5   | 5   | 9   | 8   | 9   | 10  | ..  | ..  | ..  | ..  | 1  | ..                                | Alive               | 440                                    |  |
| 94               | White..... | 136                             | ..                           | ..  | 2   | 2   | 2   | 3   | 5   | 7   | ..  | ..  | ..  | ..  | 2  | ..                                | Alive               | 440                                    |  |
| 95               | White..... | 55                              | ..                           | 5   | 6   | 9   | 10  | 11  | 6   | 9   | ..  | ..  | ..  | ..  | 2  | ..                                | Alive               | 440                                    |  |
| 96               | White..... | 82                              | ..                           | 2   | 7   | 7   | 9   | 9   | 11  | 11  | ..  | ..  | ..  | ..  | 2  | ..                                | Alive               | 440                                    |  |
| 97               | White..... | ..                              | ..                           | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | ..  | .. | ..                                | Killed              | 357                                    |  |
| 98               | White..... | 136                             | ..                           | ..  | 2   | 1   | 1   | R   | 3   | ..  | ..  | ..  | ..  | ..  | 1  | ..                                | Died                | 398                                    |  |
| Total.           |            |                                 |                              |     |     |     |     |     |     |     |     |     |     |     |    | 35                                | 2                   |                                        |  |

connective tissue was more abundant, and the papillomas became structures with stalks of loose vascular connective tissue and large connective tissue heads covered by irregular hyperkeratotic epithelium (Fig. 2). No invasiveness of the epithelium was seen.

*Regression of the papillomas.*—Cachexia usually caused rapid regression of the large papillomas, but in many rabbits regressive tendencies were seen without apparent cause. As can be seen from the tables the regressions were most frequent in the animals painted with the 120-350° C. destructive distillate. Some regressions were seen in the rabbits painted with pipe tar.

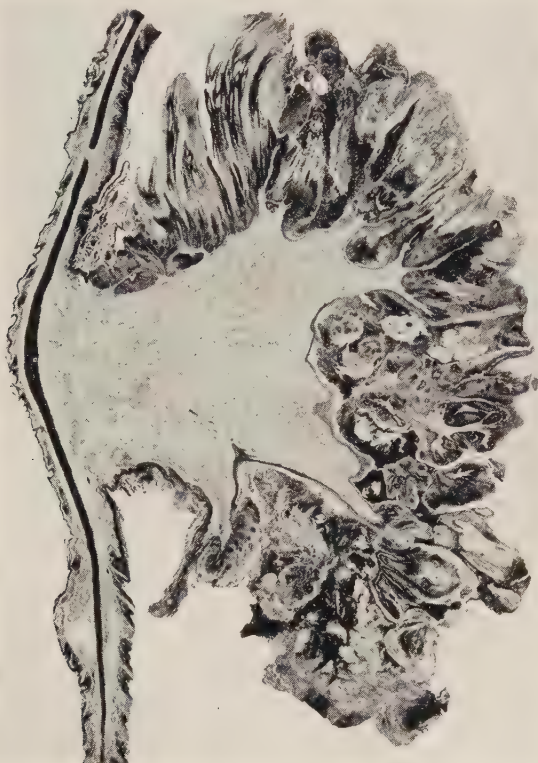


FIG. 2.—A papilloma on the ear of a rabbit (No. 43) painted with pipe tar for 211 days. Mag.  $\times 6$ .

In 6 rabbits painted with the 350-700° C. tar, however, regressive tendencies were seen after about 350 days. Histologically the regressing papillomas showed much dense collagenous tissue in their stalks, and sections taken elsewhere in the ears showed similar fibrosis between the epithelium and the cartilage. In the end stage of regression only low nubbins of tissue were seen grossly. Histologically these were small mounds of dense fibrous tissue covered by smooth but hyperkeratotic epithelium. Fibrosis of the stalk was a prominent histological feature in the regression of these tumors.

*Cause of the regression of papillomas in rabbits*

*painted with 350-700° C. destructive distillate tar.*—Something besides cachexia seemed necessary to account for the late regression of so many papillomas in these 6 rabbits painted with the 350-700° C. tar. The potency of the destructive distillate was roughly the same, as 3 white rabbits (not included in Table III) developed papillomas in 59, 59, and 84 days. A fourth killed on the 84th day had no tumors.

In the rabbits in which regressions of tumors were taking place there did not seem to be any systemic resistance to further papilloma formation, as in 4 of the 6 rabbits in which regressions were occurring on the inside of the right ear, small papillomas were appearing where the tar was accidentally rubbed on the backs of the right and left ears.

This lack of systemic resistance was confirmed by painting the insides of the left ears of 2 of these rabbits with this tar from the 540th day on, the painting of the right ears being continued. Multiple papillomas appeared on the inside of the left ear of rabbit No. 21 on the 42nd day, and of rabbit No. 31 on the 62nd day (not included in Table III), although in rabbit No. 21 the tumors on the right ear continued to regress and in rabbit No. 31 the right ear remained without tumors despite the continued painting.

It appears probable that the regression of the papillomas, when not caused by cachexia, is caused by some local change in the papillomas themselves accompanied by resistance of the surrounding skin to further papilloma formation. It seems probable that this is due to fibrosis, and not to a general resistance of the rabbit to the papilloma stimulating action of the tar.

*Production of carcinomatoid tumors.*—Out of a total of 156 tumors examined histologically in the rabbits painted with tobacco tars, 12 were carcinomatoid tumors, the rest papillomas.

A typical example of these tumors was one seen in a biopsy of the ear of rabbit No. 30 painted with 350-700° C. tar. This tumor was removed on the 229th day along with 1 large and 4 small papillomas. This carcinomatoid was a low rounded tumor 7 mm. across and 2.5 mm. high. The time of its appearance was unknown. Histologically the epithelium on the surface of the tumor was hyperkeratotic, and thin strands of epithelium projected down into the connective tissue (Fig. 3). On serial sections groups of cells lay free in the connective tissue and others were growing in lymphatic vessels (Fig. 4). All these cells were well keratinized and not anaplastic or hyperchromatic. This carcinomatoid was probably completely excised. The subauricular node, removed at the same time, was normal histologically. At death at 521 days only 8 papillomas and 5 regressed papillomas were found. The subauricular lymph node and lungs were normal.

Three other similar carcinomatoid tumors were found on other rabbits painted with 350-700° C. tar.



One tumor was biopsied on rabbit No. 29, on the 485th day, and grew slowly during the last 49 days of life. Both the first specimen and the tumor removed at death showed local intralymphatic growth. A small carcinomatoid tumor was found on the ear of rabbit No. 25 at death at 593 days. Its tumor cells had formed many epithelial pearls which were encased by dense connective tissue. Some well keratinized cells

last 70 days. At autopsy the tumor was a firm rounded nubbin 1 cm. in diameter and grossly looked much like the regressed papillomas described above. On section the tumor was 1 cm. long and 6 mm. thick (Fig. 5). It was composed of moderately anaplastic squamous cells. These were slightly hyperchromatic, with deep staining nuclei and pale eosinophilic cytoplasm. A few mitotic figures were found. The cells, in small

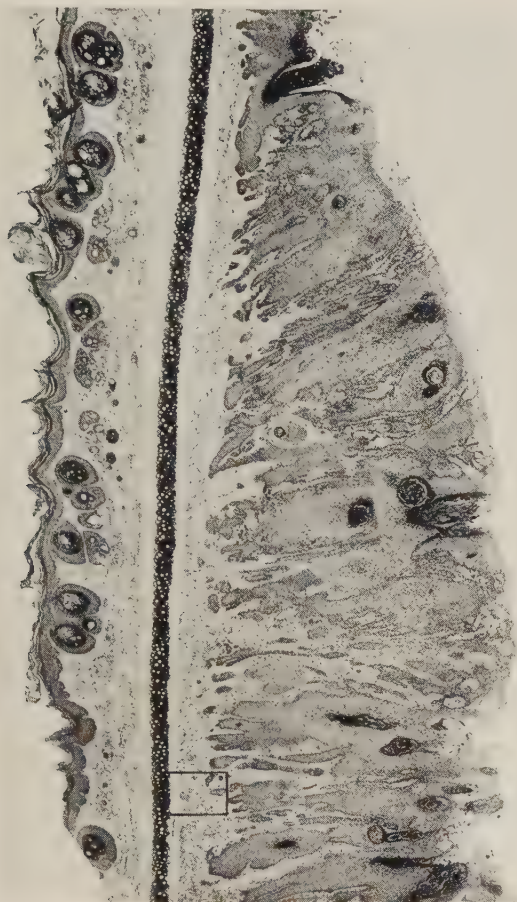


FIG. 3.—A carcinomatoid tumor on the ear of a rabbit (No. 30) painted with the 350-700° C. destructive distillate tar of tobacco for 229 days. Mag.  $\times 22$ .

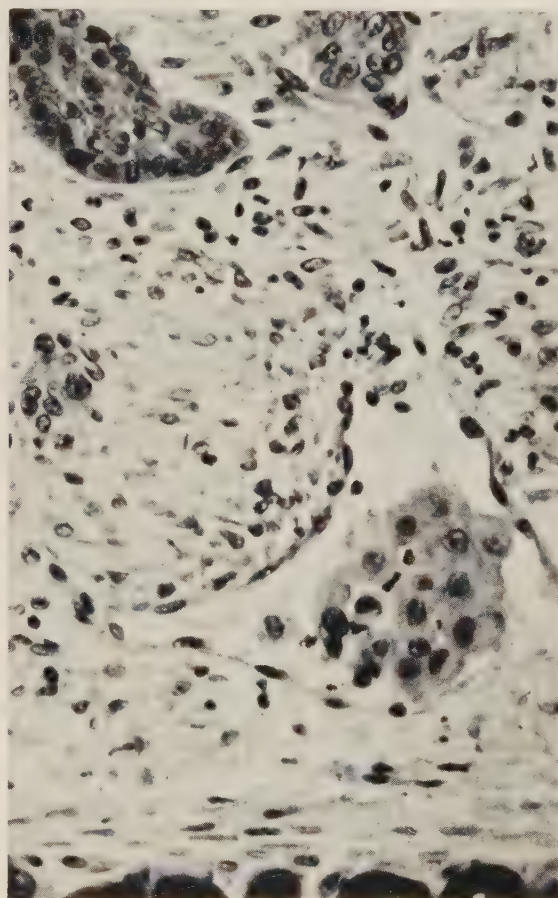


FIG. 4.—A higher magnification of the small area in Fig. 3 enclosed by a rectangle, showing a group of squamous epithelial cells in a lymphatic vessel. Serial sections showed this to be a cord of tumor cells. Mag.  $\times 375$ .

had invaded local lymphatics. The subauricular node and lungs contained no tumor cells.

Rabbit No. 21 is still living. A biopsy at 650 days showed a carcinomatoid tumor with local lymphatic invasion. This tumor has been growing slowly for 90 days. The subauricular node is not enlarged.

Only one of the carcinomatoid tumors examined was anaplastic. A second carcinomatoid tumor was discovered on rabbit No. 29 at the time of the animal's death. It had first appeared as a papilloma near the tip of the ear at 167 days. It remained as a papilloma for about 200 days, then seemed to regress during the

nests and threads, formed many small epithelial pearls. The tumor apparently arose on the inner side of the ear and extended around the tip to the other side of the ear. The tumor was focally ulcerated. The tumor cells had invaded the connective tissue more extensively than most carcinomatoids; many tumor cells lay just above the cartilage, and in one place grew through a small defect in the cartilage. Near one margin the tumor cells had extended under intact epithelium, and also into the connective tissue and the lymphatic vessels about a large vein and several nerves (Fig. 6). This



tumor had the local appearance of a squamous cell carcinoma, but has been classified as a carcinomatoid tumor since no metastases were found in either the subauricular lymph node or lungs.

Five carcinomatoid tumors were found in the rabbits painted with the 120-350° C. destructive distillate tar. Two of these were found on rabbit No. 2, which died on the 127th day. Six benign papillomas and 2 car-

diameter. These 3 growths had been present for 220 days and had grown very little, remaining as low small tumors. Histologically all were carcinomatoids. One showed infiltration of epithelial cells into the connective tissue in threads and clusters of cells, the other 2 showed a more invasive tendency, with intralymphatic growth of tumor cells. These last 2 tumors also showed more epithelial pearls than the first. All 3 were superficially ulcerated. The lungs contained no tumor cells. In two sections of the subauricular lymph node, lying in one of the lymph sinuses entering the

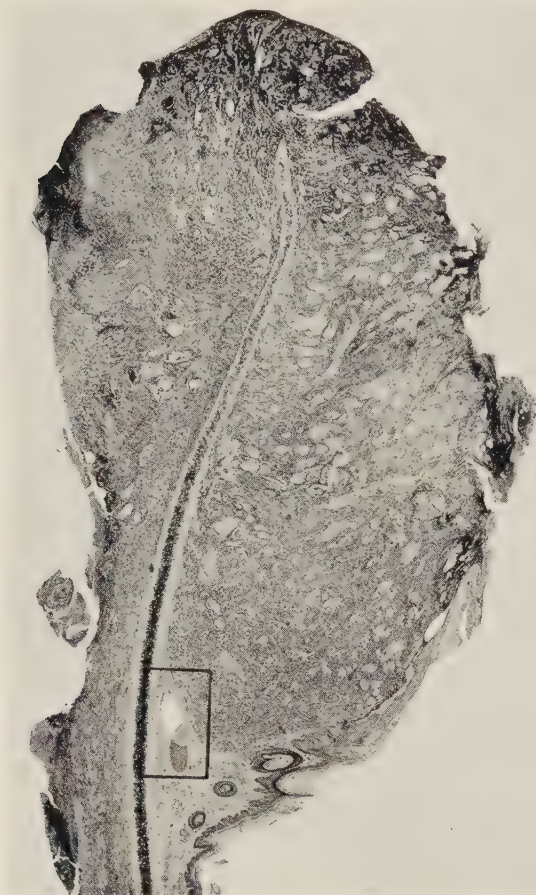


FIG. 5.—An anaplastic carcinomatoid tumor on the ear of a rabbit (No. 29) painted with the 350-700° C. destructive distillate tar of tobacco for 534 days. The small circular areas are epithelial pearls. Note the growth of the tumor along the cartilage plate and also the growth under intact epithelium. Mag.  $\times 7$ .

cinomatoids were found. Both were typical infiltrative flat tumors, and both showed local invasion of the lymphatic spaces. The subauricular node and lungs contained no tumor.

What was probably a tumor embolus was found in the lymph node of a rabbit painted with this 120-350° C. tar. Rabbit No. 6 died on the 376th day. One of the tumors was a small papilloma, the other 3 flat warty growths measuring 10, 8, and 8 mm. in

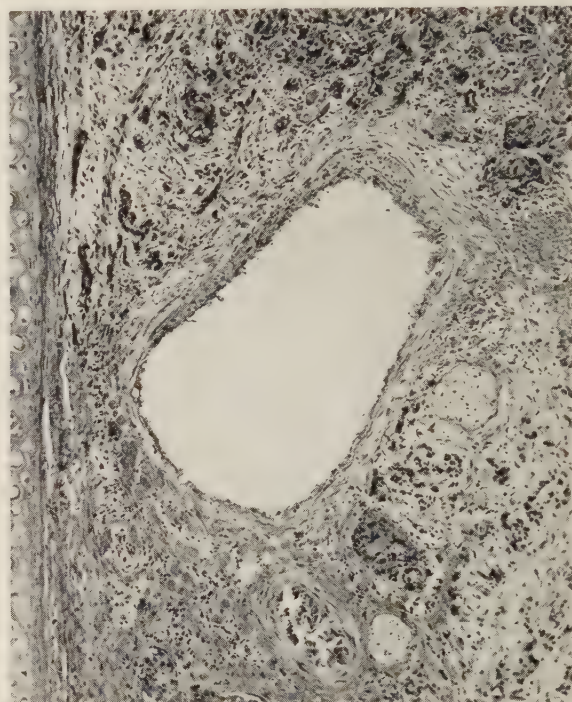


FIG. 6.—A higher magnification of an area similar to that enclosed by a rectangle in Fig. 5, but from another section of the same tumor. The tumor cells are invading the connective tissue and lymphatics about the large vein and the nerves. Mag.  $\times 90$ .

node, was a cluster of what probably were keratinized epithelial cells. The nuclei were large and pale, the cytoplasm was very eosinophilic, and the cell borders were sharp. No intracellular bridges could be made out. Serial sections showed no similar cell clusters, and no such groups of cells have been seen in any of the other lymph nodes studied. If this was, as seems probable, a group of tumor cells, it must be considered as an embolus to the lymph node and not as a definite metastasis.

Rabbit No. 41, painted with pipe tar, died after 135 days with 2 tumors, one a small papilloma, the other a carcinomatoid tumor 3 mm. across showing definite invasiveness. No metastases were found. This tumor



had been present for 73 days as a flat tumor, which not only did not grow, but even became smaller before death.

The second carcinomatoid in the rabbits painted with pipe tar was found on rabbit No. 54 at death after 232 days, along with a papilloma. This carcinomatoid tumor was also small, with local lymphatic invasion (Fig. 7). The subauricular lymph node and

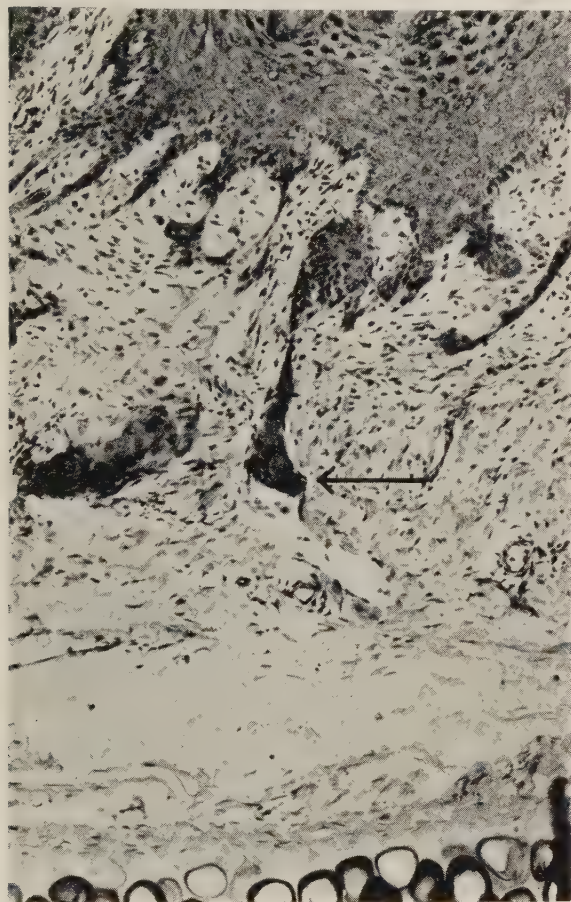


FIG. 7.—A portion of a carcinomatoid tumor produced on a rabbit (No. 54) by painting pipe tar for 232 days. The arrow points to a group of tumor cells growing in a local lymphatic. Mag.  $\times 145$ .

lungs contained no tumor cells. This carcinomatoid had grown very slowly for 44 days.

Twenty-nine biopsies have been made of all the possibly carcinomatoid tumors on the ears of the 14 rabbits painted with pipe tar that are still alive. Although these animals had been painted for from 425 to 629 days, all of the biopsied tumors were papillomas.

Five carcinomatoid tumors were seen in rabbits painted with fractional distillates, and are discussed under that heading.

A total of 17 of these locally invasive carcinomatoid

tumors was seen in 11 rabbits. The first of these was found after 127 days of painting. Another which subsequently proved to be a carcinomatoid probably arose on the 62nd day. Many arose before 200 days, but some as late as 600 days. None of the tumors grew rapidly, most slowly. In 8 out of 11 rabbits the epithelial cells of the tumors invaded the local lymphatics. Out of the 11 rabbits, 10 have died and no distant metastases have been found, although what was probably a tumor embolus to a lymph node was found.

*Causes of death.*—Of the rabbits painted with the 350-700° C. destructive distillate tar, 10 rabbits died after varying periods of cachexia. Six had pneumonia with abscesses, 2 pyemia, 1 urinary tract infection, and 1 paralysis. No visceral lesions attributable to the tar were observed.

The 120-350° C. tar often caused convulsive symptoms if painted too freely. Ten of the 16 rabbits died a few hours after painting, apparently from acute nico-

TABLE VI: FRACTIONAL DISTILLATES OF THE 350-700° C. DESTRUCTIVE DISTILLATE TAR AT 10 TO 13 MM. HG. PRESSURE

| Temperature of liquid in still | Quantity  | Color       | Consistency |
|--------------------------------|-----------|-------------|-------------|
| 30-100° C.                     | 60.3 gm.  | Yellow      | Watery      |
| 100-140° C.                    | 77.9 gm.  | Brown       | Oily        |
| 140-180° C.                    | 84.4 gm.  | Deep brown  | Viscid      |
| 180-220° C.                    | 80.2 gm.  | Deep brown  | Semisolid   |
| 220-240° C.                    | 59.3 gm.  | Brown-black | Semisolid   |
| Residue                        | 181.3 gm. | Black       | Solid tar   |

tine poisoning. Two others died of pneumonia, 2 of diarrhea, and 2 of cachexia without focal lesions.

Fourteen of the rabbits painted with pipe tar are still alive and being painted. Of the 10 that have died, 5 died acutely of what probably was nicotine poisoning, 1 of pneumonia, 1 of an unknown debilitating disease, and 2, Nos. 56 and 97, were killed.

*Fractional distillates of 350-700° C. tar; preparation of distillates.*—Since it seemed probable that some chemical substance was the cause of the papilloma-producing action, a fractional distillation was done on the pooled 350-700° C. destructive distillates of 20 kg. of tobacco, 625 gm. of tar in all.

Water and the lower alcohols were first removed by heating to 120° C. in a glycerine bath at atmospheric pressure, leaving 567 gm. of tar. This tar was heated in a glass still at a pressure of from 10 to 13 mm. Hg over first a glycerine, then a solder bath. The quantities obtained are shown in Table VI.

*Effect of fractions of tar on rabbits.*—Each of 5 fractions and the residue was tested. Two cubic centimeters of benzene were added as a solvent to each gram of substance. All rabbits used were white. All except 2 of the rabbits were painted for at least 186, and most for 200 days. One in the 140-180° C. series

died at 96 days and one in the residue series died at 147 days, both without tumors. The results are shown in Table VII.

It was apparent that the 220-240° C. substance was the most active fraction, but this was only a crude estimate, and the important fact was that the active fraction boiled above about 200° C. at a pressure of

TABLE VII: TUMORS PRODUCED BY FRACTIONAL DISTILLATES OF 350-700° C. DESTRUCTIVE DISTILLATE TAR

| Fraction at 10-13 mm. Hg | Total rabbits | Rabbits with tumors | Time of development in days | Total tumors seen |
|--------------------------|---------------|---------------------|-----------------------------|-------------------|
| 30-100° C.....           | 4             | 0                   |                             | 0                 |
| 30-100° C.....           | 4             | 0                   |                             | 0                 |
| 100-140° C.....          | 4             | 0                   |                             | 0                 |
| 140-180° C.....          | 5             | 0                   |                             | 0                 |
| 180-220° C.....          | 4             | 1                   | 136                         | 1                 |
| 220-240° C.....          | 4             | 4                   | 34, 52, 83, 93              | 27                |
| Residue.....             | 4             | 3                   | 41, 117, 171                | 9                 |

TABLE VIII: SUMMARY OF TUMORS PRODUCED ON RABBITS BY TOBACCO TARS

| Substance used                             | Number rabbits | Per cent developing tumors | Mean time, in days | Av. no. per ear | No. tumors studied histologically | Papillomas | Carcinomatoids |
|--------------------------------------------|----------------|----------------------------|--------------------|-----------------|-----------------------------------|------------|----------------|
| 350-700° C. destructive distillate tar.... | 12             | 100                        | 57                 | 9.0             | 73                                | 68         | 5              |
| 120-350° C. destructive distillate tar.... | 17             | 94                         | 75                 | 4.3             | 45                                | 40         | 5              |
| Pipe tar.....                              | 24             | 92                         | 108                | 4.7             | 38                                | 36         | 2              |
| Fractions of the 350-700° C. tar.....      |                |                            |                    |                 | 27                                | 22         | 5              |
| Total .....                                |                |                            |                    |                 | 183                               | 166        | 17             |

10 to 12 mm. Hg. It was evident that the lower boiling substances were not active.

*Types of tumors produced.*—Of 27 tumors examined histologically 22 were papillomas, and 5 carcinomatoids. Two of the carcinomatoid tumors were seen in rabbits painted with the residue. One appeared after 165 days and was only 3 mm. across when the animal was killed on the 195th day. The other was an 8 mm.

carcinomatoid found among 7 papillomas in a rabbit dying after 508 days. Neither carcinomatoid tumor had invaded lymphatics, and the subauricular nodes and lungs of these rabbits showed no tumor.

The other 3 carcinomatoid tumors were in a rabbit painted with the 220-240° C. fraction and killed at 224 days. One of these tumors appeared on the 92nd day and grew very slowly. At death it was only 5 mm. across and 2 mm. high. The other 2 appeared later, and also grew slowly. Two of the 3 showed local lymphatic invasion, but the subauricular nodes and lungs were normal.

*Attempt to demonstrate a virus in the papillomas.*—The method described by Shope (50) was used, and 2 filtrates made, one from papillomas on rabbits in the 350-700° C. series, the other from papillomas on rabbits in the pipe tar series. One filtrate was inoculated on a scarified strip on the right side of the abdomen of 5 white rabbits, the other on the left side. Other rabbits were injected intramuscularly and intravenously with the filtrates. No tumors developed, and all animals were killed after 60 days.

*Effect of injection of the 350-700° C. destructive distillate tar into rats.*—One group of white rats was injected intrascapularly with 0.05 cc. of the crude tar. Thirteen survived the immediate toxic effects, and 6 lived 6 months, 2 lived 12 months, and 1 lived 18 months. Another group of rats was similarly injected with 1 cc. of denicotinized tar. Seventeen rats survived the immediate effects, 15 lived 6 months, and 10 are alive at 12 months.

No tumors have been seen in either group. In those which have died, black pigment and oily material in macrophages were seen at the injection site.

*Effects of the tobacco tars on mice.*—All mice were painted on the back 5 times a week. The 350-700° C. destructive distillate tar was not very toxic and was painted on mice both in the original form used on rabbits, and after denicotinization. Both the 120° C. and 350° C. destructive distillate tar and the pipe tar had to be denicotinized before being painted on mice, as in their original form they caused convulsions and death in most mice.

TABLE IX: TUMORS PRODUCED ON MICE BY TOBACCO TARS

| Substance used                                          | Grams per mouse per painting | Number of mice alive |    |    |    |    |    |    | Papillomas |                 | Carcinomas |                 |
|---------------------------------------------------------|------------------------------|----------------------|----|----|----|----|----|----|------------|-----------------|------------|-----------------|
|                                                         |                              | Months               |    |    |    |    |    |    | No.        | Time, in months | No.        | Time, in months |
| 350-700° C. destructive distillate tar.....             | 0.015                        | 30                   | 29 | 27 | 15 | 7  | 1  | .. | 4          | 7.5 to 11.5     | ..         | ..              |
| Denicotinized 350-700° C. destructive distillate tar... | 0.018                        | 46                   | 44 | 30 | 8  | 2  | .. | .. | 8          | 7.5 to 11       | 1          | 8.5             |
| Denicotinized 120-350° C. destructive distillate tar... | 0.015                        | 76                   | 68 | 55 | 24 | 17 | 5  | 4  | 1          | 14              | ..         | ..              |
| Mixture of watery parts of distillates .....            | 0.20                         | 30                   | 30 | 30 | 26 | 19 | 11 | 1  | ..         | ..              | ..         | ..              |
| Denicotinized pipe tar.....                             | 0.013                        | 60                   | 58 | 53 | 40 | 32 | 28 | 20 | 2          | 14              | 1          | 17              |



A group of mice were painted with a mixture composed of equal parts of the watery portions of the 0-120° C., the 120-350° C., and the 350-700° C. destructive distillates.

The details of the experiments are shown in Table IX.

The destructive distillate tars produced baldness after a few paintings, and eventually both the pipe tar and

8.5 months, at a time when 14 mice were alive in this series. This carcinoma arose at the painting site and grew rapidly for the month before the animal was killed. Histologically the tumor was typical (Fig. 8) and metastasized to an adjacent lymph node. This denicotinized tar seemed to be more toxic than the crude 350-700° C. tar, but produced more papillomas and the only malignancy.

The denicotinized pipe tar also produced one malignant tumor on a mouse. This tumor arose at the painting site during the 17th month when 22 mice were alive in this series. It grew rapidly and soon had an ulcerated center and raised pearly edges. Histologically the tumor was an anaplastic squamous cell carcinoma, with many mitotic figures. It did not metastasize. The remaining mice show no tumors.

#### DISCUSSION

*Tumors of rabbits' ears produced by tarring.*—Papillomas and carcinomatoid tumors, but no proved carcinomas have been produced in rabbits by painting the destructive distillates of tobacco and pipe tars. The papillomas are essentially the same as the folliculo-epitheliomas of Yamagiwa and Ichikawa (57), and the common papillomas of Rous and Kidd (44).

The significance of the production of these benign papillomas in rabbits, both in these experiments and the experiments of others, is difficult to evaluate. In general these papillomatous growths are produced by known carcinogenic agents before the true carcinomas appear, but these benign tumors have not been proved to be any indication of the carcinogenic activity of the substance producing them.

In these experiments 17 carcinomatoid tumors have been seen in 11 rabbits. These tumors were definitely locally invasive, infiltrating as threads of epithelial cells both into the connective tissue and into the local lymphatic vessels. Epithelial pearls were seen occasionally. Growth through the cartilage plate and under intact epithelium occurred in one tumor. Their morphology was similar to that of a well differentiated squamous cell carcinoma. No distant metastases occurred, although in one instance what seemed to be a tumor embolus reached a subauricular lymph node. Similar tumors have been observed by others, but conclusions as to their character have varied.

Rous and Kidd (44) noted that the local invasive qualities of carcinomatoids induced by coal tar in rabbits were dependent on the continuation of tarring. These tumors did not have the unrestricted growth properties of a malignant neoplasm, and did not metastasize to distant sites. Despite long continued tarring these tumors eventually took on gross forms of old papillomas, or retrogressed. No carcinoid tumors became carcinomas.

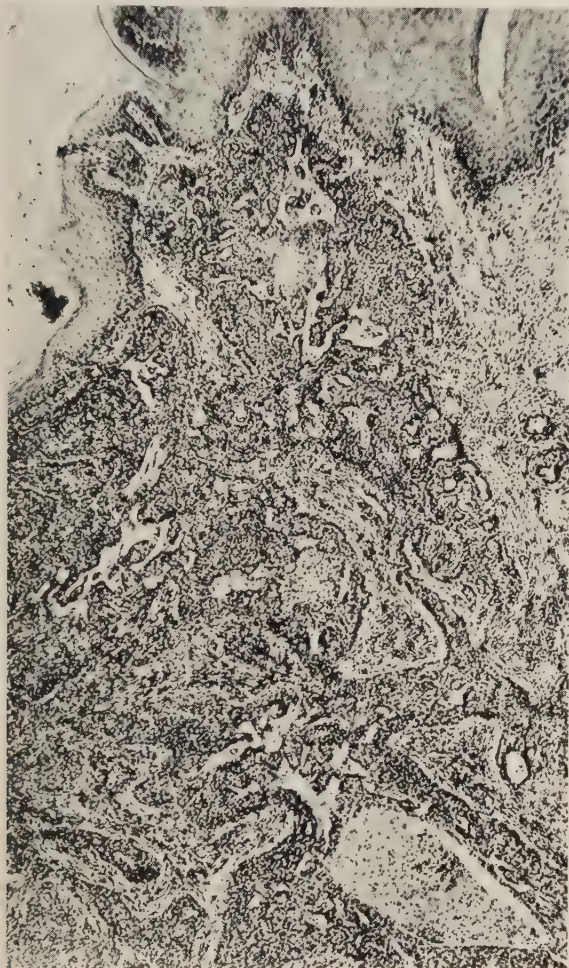


FIG. 8.—The squamous cell carcinoma produced on a mouse by painting the denicotinized 350-700° C. destructive distillate tar of tobacco for 300 days. The tumor is rather anaplastic, with cells growing in sheets and cords, but not forming typical epithelial pearls. A metastasis to an adjacent lymph node is well keratinized. Mag.  $\times 75$ .

the watery mixture from the distillates caused partial baldness.

Papillomas were produced frequently by the 350-700° C. tars. All papillomas were verified histologically. Frequently inflammatory hyperplasias were mistaken grossly for papillomas.

The denicotinized 350-700° C. destructive distillate tar produced a squamous cell carcinoma in a mouse at

In 1932 Roffo (32, 33) reported the production of one carcinoma with distant metastases. His subsequent papers describe and illustrate lesions produced by tobacco tars which he interpreted as carcinomas, none of which metastasized to distant sites. Obviously some of these were anaplastic squamous cell carcinomas invading and destroying the ear. However other tumors which he called carcinomas seem to be of the type called carcinomatoids by Rous and Kidd. As far as the production of papillomas and locally invasive carcinomatoid tumors are concerned, both by the application of the destructive distillate tars and the pipe tar, this study entirely confirms the work of Roffo. The main difference seems to be a matter of interpretation.

It is impossible to re-examine the earlier specimens of tar cancers in rabbits, such as those observed in the epoch-making study of Yamagiwa and Ichikawa (57). These workers obtained only a very few carcinomas which metastasized, and Rous and Kidd were convinced that many tumors which Yamagiwa and Ichikawa called carcinomas were probably carcinomatoids. Evidently it is difficult to induce cancers in rabbits by tarring which fulfill all of the criteria of malignancy.

Even with as potent a carcinogenic agent as benzo(a)pyrene the rate of carcinoma production in rabbits is low (6, 46, 49), and few metastases from what have been called carcinomas have been reported. However it is important to note that when a very active carcinogen is painted on wild cottontail rabbits' ears true squamous cell carcinomas are produced, which give rise to many distant metastases, as shown by Syverton and Berry (53), who used methylcholanthrene. The production of carcinomas of the skin of a wild rabbit's ear does not seem to be difficult with methylcholanthrene.

What then is the status of these carcinomatoid tumors? In this work with tobacco tars evidence does not indicate what the ultimate fate of these tumors would have been. Most of the tumors were identified only at autopsy, although some were seen very early in the painting period. It is of importance to note the absence of definite metastases in all 11 animals with carcinomatoid tumors. It was not proved that such tumors could produce distant metastases. In view of the work of Rous and Kidd it would seem likely that the invasive behavior of these tumors depended on the repeated application of an extrinsic stimulus rather than on an intrinsic capacity for unrestricted growth. There is no evidence that the production of these carcinomatoid tumors in rabbits is an indication of carcinogenic activity of the tobacco tars.

*Carcinogenic activity of tobacco tars in mice.*—Although the stock mice used in these experiments readily developed squamous cell carcinomas of the skin when painted with coal tar of known carcino-

genicity, only 2 squamous cell carcinomas developed as the result of painting animals of the same stock with tobacco tars. Both tumors arose at the sites of painting. One developed after painting with the denicotinized 350-700° C. destructive distillate tar for 8.5 months, the other after 17 months' application of denicotinized pipe tar. Since no similar spontaneous tumors had been seen in some 2,000 of these mice, the production of these squamous cell carcinomas indicated that both tars had a low but definite carcinogenic activity. The earlier time of cancerization, as well as the greater capacity to produce papillomas, indicated that the activity of the 350-700° C. destructive distillate was greater than that of pipe tar. Both tobacco tars were a great deal less active than the potent coal tar used in the control experiments.

These observations are in accordance with those of Cooper, Lamb, and Sanders (11), Campbell (7, 8), and Sugiura (52), but the work of Taki (20) indicated a higher carcinogenic activity of pipe tar on mice.

#### SUMMARY AND CONCLUSIONS

Twelve rabbits were painted on the ears with a tar produced by the destructive distillation of tobacco at from 350-700° C. Between the 49th and 79th day all rabbits developed tumors at the painting site. The rabbits lived from 238 to over 660 days. Sixty-eight of the tumors examined histologically were papillomas, and 5 were carcinomatoid tumors. No carcinomas were produced. A 130-350° C. destructive distillate tar produced tumors in 16 out of 17 rabbits, but more slowly than the 350-700° C. tar. Forty of these tumors were papillomas and 5 carcinomatoid tumors.

Twenty-four rabbits were painted with tar obtained by smoking tobacco in pipes. Tumors were produced in from 37 to 374 days in 22 out of the 24 rabbits. Seven animals have lived over 640 days. Thirty-six tumors examined histologically were papillomas and 2 were carcinomatoid tumors. No carcinomas were produced.

These tars produced tumors less readily when painted on mice, but the 350-700° C. tar (denicotinized) produced 8 papillomas and 1 squamous cell carcinoma which arose at 8.5 months when 14 mice were alive. The pipe tar (denicotinized) produced 2 papillomas and 1 squamous cell carcinoma, which arose at 17 months when 22 mice in this series were alive.

The significance of these results is discussed.

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REFERENCES

1. ABBE, R. Cancer of the Mouth; The Case Against Tobacco. New York State J. Med., **102**:1-5. 1915.
2. BLOODGOOD, J. C. Cancer of the Tongue; A Preventable Disease. J. A. M. A., **77**:1381-1387. 1921.
3. BOGEN, E., and R. N. LOOMIS. Tobacco Tar; Experimental Investigation of its Alleged Carcinogenic Action. Am. J. Cancer, **16**:1515-1521. 1932.
4. BRODERS, A. C. Squamous-Cell Epithelioma of the Lip. J. A. M. A., **74**:656-664. 1920.
5. BROSCH, A. Theoretische und experimentelle Untersuchungen zur Pathogenesis und Histogenesis der Malignen Geschwülste. Virchows Arch. f. Path. Anat., **162**:32-85. 1900.
6. BRUNSCHWIG, A., D. TSCHETTER, and A. HAMANN. Observations on Injections of Human Wart (Verruca Vulgaris) Extracts into Rabbits with Benzpyrene Papillomas on the Ear. Am. J. Cancer, **38**:50-54. 1940.
7. CAMPBELL, J. A. Effects of Exhaust Gases From Internal Combustion Engines and of Tobacco Smoke upon Mice, with Special Reference to Incidence of Tumors of the Lung. Brit. J. Exper. Path., **17**: 146-158. 1936.
8. CAMPBELL, J. A. Carcinogenic Agents Present in the Atmosphere and Incidence of Primary Lung Tumors in Mice. Brit. J. Exper. Path., **20**:122-132. 1939.
9. CHIKAMATSU, T. Künstliche Erzeugung der Krebs durch Tabakteer bei Kaninchen und Maus. Tr. Jap. Path. Soc., **21**:244. 1931.
10. COOK, J. W., G. A. HASLEWOOD, C. L. HEWETT, I. HIEGER, E. L. KENNAWAY, and W. V. MAYNEORD. Chemical Compounds as Carcinogenic Agents. Am. J. Cancer, **29**:219-259. 1937.
11. COOPER, E. A., F. W. M. LAMB, E. SANDERS, and E. L. HIRST. Role of Tobacco Smoking in Production of Cancer. J. Hyg., **32**:295-300. 1932.
12. EWING, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 4th Ed. pp. 527, 879, 913. 1940.
13. HELWIG, F. C. Growth-Producing Effects of Extracts of Tobacco on Mice. J. A. M. A., **91**:150-151. 1928.
14. HIEGER, I. Fluorescence Spectrum of 3:4-Benzpyrene. Am. J. Cancer, **29**:705-714. 1937.
15. HOFFMAN, E., H. T. SCHREUS, and E. ZURHELLE. Experimental Tar Tumors. Deutsche med. Wchnshr., **49**:633-624. 1923.
16. HOFFMAN, F. L. Cancer and Smoking Habits. Ann. Surg., **93**:50-67. 1931.
17. HOFFMAN, F. L. Cancer and Diet. Williams and Wilkins Co., Baltimore, 1937.
18. KENNAWAY, E. L. Experiments on Cancer-Producing Substances. Brit. M. J., **2**:1-4. 1925.
19. KENNAWAY, E. L., and B. SAMPSON. Tumors of Skin and Mammary Gland Caused by Pyrogenous Products of Cholesterol. J. Path. & Bact., **31**:609-612. 1928.
20. KINOSHITA, R. Studies on Carcinogenic Chemical Substances. Tr. Jap. Path. Soc., **27**:665-725. 1937.
21. LU-FU-HUA. Über die Erzeugung von Krebs durch Tabakteerpinselung beim Kaninchen. Frankfurt. Ztschr. f. Path., **46**:513-522. 1934.
22. LU-FU-HUA. Über die Erzeugung von Krebs durch Tabakteerpinselung beim Kaninchen; über das Solitärauftreten einzelner Tumoren auf einer diffus gereizten Körperstelle. Frankfurt. Ztschr. f. Path., **47**:52-62. 1934.
23. LICKINT, F. Tabak und Tabakrauch als ätiologischer Faktor des Carcinoms. Ztschr. f. Krebsforsch., **30**:349-365. 1929.
24. LICKINT, F. Der Bronchialkrebs der Raucher. München. med. Wchnshr., **82**:1232-1234. 1935.
25. McNALLY, W. D. Tar in Cigarette Smoke and its Possible Effects. Am. J. Cancer, **16**:1502-1514. 1932.
26. MIESCHER, G., F. ALMASY, and K. KLÄUI. Fluorescence Spectroscopic Determinations of 1:2-Benzpyrene. Biochem. Ztschr., **287**:189-197. 1937.
27. MORATTI, A. Metaplasia malpighiana sperimentale della mucosa gastrica di cane. Tumori, **6**:101-139. 1932.
28. MÜLLER, F. H. Tabakmissbrauch und Lungencarcinom. Ztschr. f. Krebsforsch., **49**:57-85. 1939.
29. ROFFO, A. H. Leucoplasie tabáquica experimental. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **7**: 130-144. 1930.
30. ROFFO, A. H. Desarrollo de un carcinoma en el conejo par el tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **7**:501-538. 1930.
31. ROFFO, A. H. Durch Tabak beim Kaninchen entwickeltes Carcinom. Ztschr. f. Krebsforsch., **33**:321-332. 1931.
32. ROFFO, A. H. Carcinome développé chez un lapin après trois ans, par l'effet du tabac. Neoplasmes, **11**:77-89. 1932.
33. ROFFO, A. H. Carcinoma desarrollado en un conejo fumador a los tres años. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **8**:545-573. 1931.
34. ROFFO, A. H. El tabaco como cancerígeno. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **13**:287-333. 1936.
35. ROFFO, A. H. Der Tabak als Krebserzeugendes Agens. Deutsche med. Wchnshr., **63**:1267-1271. 1937.
36. ROFFO, A. H. Espectrografia de los derivados obtenidos por destilación directa de los tabacos y su relacion como agentes cancerígenos. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **14**:311-399. 1937.
37. ROFFO, A. H. El tabaco rubio como cancerígeno. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **15**:5-18. 1937.
38. ROFFO, A. H. Unidad cancerígena de los alquitranes des diversos tipos de tabacos. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **15**:349-406. 1938.
39. ROFFO, A. H. Le goudron de tabac cancérigène; unité cancérigène des goudrons de divers types de tabac. Acta, Union internat. contre cancer, **4**:755-762. 1939.
40. ROFFO, A. H. 1:2-benzopirene cancerígeno extraído del alquitrán del tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **16**:1-38. 1939.
41. ROFFO, A. H. Krebserzeugendes Benzpyren, gewonnen aus Tabakteer. Ztschr. f. Krebsforsch., **49**:588-597. 1939.
42. ROFFO, A. H. Sobre los filtros en el tabaquismo el narguíté y el algodón como filtro del alquitrán de tabaco. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **16**: 255-268. 1939.
43. ROFFO, A. H. Krebserzeugende Einheit der verschiedenen Tabakteere. Deutsche med. Wchnshr., **65**:963-967. 1939.
44. ROUS, P., and J. G. KIDD. Comparison of Virus-Induced Rabbit Tumors with Tumors of Unknown Cause Elicited by Tarring. J. Exper. Med., **69**:399-424. 1939.
45. ROUS, P., and J. G. KIDD. Activity, Transforming, and Carcinogenic Effects of Rabbit Papilloma Virus (Shope) upon Implanted Tar Tumors. J. Exper. Med., **71**:787-812. 1940.
46. SCHÜRCH, O., and A. WINTERSTEIN. Über die krebserregende Wirkung aromatischer Kohlenwasserstoff. Ztschr. f. physiol. Chem., **236**:79-91. 1935.
47. SCHÜRCH, O., and A. WINTERSTEIN. Experimentelle Untersuchungen zur Frage Tabak und Krebs. Ztschr. f. Krebsforsch., **42**:76-92. 1935.

48. SCHÜRCH, O., and A. WINTERSTEIN. Experimenteller Beitrag zur Frage Tabak und Krebs. *Ztschr. f. Krebsforsch.*, **46**:414-419. 1937.
49. SCHÜRCH, O. Zur krebserzeugenden Wirkung des Benzpyren beim Kaninchen. *Ztschr. f. Krebsforsch.*, **49**:353-362. 1939.
50. SHOPE, R. E., and E. W. HURST. Infectious Papillomatosis of Rabbits with Note on Histopathology. *J. Exper. Med.*, **58**:607-624. 1933.
51. SIMONDS, E. J. Primary Carcinoma of the Lung. Year Book Publishers Inc., Chicago, 1937.
52. SUGIURA, K. Observations on Animals Painted with Tobacco Tar. *Am. J. Cancer*, **48**:41-49. 1940.
53. SYVERTON, J. T., and G. P. BERRY. Sarcomata and Carcinomata Induced in Cottontail Rabbits by Methylcholanthrene. *Science*, **91**:298. 1940.
54. WACKER, L., and A. SCHMINCHE. Experimentelle Untersuchungen zur kausalen Genese atypischer Epithelwucherungen. *München. med. Wchnschr.*, **58**:1607-1613. 1911.
55. WACKER, L., and A. SCHMINCHE. Experimentelle Untersuchungen zur kausalen Genese atypischer Epithelwucherungen. *München. med. Wchnschr.*, **58**:1680-1682. 1911.
56. WATSON, A. F. Experimental Skin Tumors in Rat Produced by Tar. *J. Path. & Bact.*, **36**:251-256. 1933.
57. YAMAGIWA, K., and K. ICHIKAWA. Experimental Study of the Pathogenesis of Carcinoma. *J. Cancer Research*, **3**:1-21. 1918.







